Digital microfluidic chip technology for cytotoxicity studies on yeast cells at spatio-temporal resolution

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Elucidating the heterogeneous responses of single cells to external perturbation is one of the central interests in cell biology. However, continuous monitoring of non-adhering single cells is still hindered by the limited availability of systems for precise manipulation and retention of cells at a desired location. Flow cytometry is one of the most widely used techniques to characterize non-adhering cells, however, its inability to follow the response of single cells in time is a major drawback[1]. **Here we describe an application of electrowetting-on-dielectric (EWOD) based digital microfluidic (DMF) chip[2], as a platform for trapping of non-adhering cells in an array of microwells. Consequently, the single cell response to a drug incubation can be monitored with a spatio-temporal resolution in an automatic way.**

The scheme of DMF device for trapping single yeast cells (*Saccharomyces cerevisiae*) is illustrated in Fig.1. The device consists of two parallel glass plates, namely the actuation plate and grounding plate (Fig.1a). An array consisting of 22000 microwells (5 µm wide and 3 µm deep) was fabricated in the Teflon layer of the grounding plate[3]. The microwells have hydrophilic bottom and hydrophobic sidewalls (Fig.1a,inset). In order to trap yeast cells, the cell droplet was transported over the array for multiple times, referred to as seeding cycles (Fig.1a), resulting in entrapment of single cells in femtoliter droplets in the microwells. These spatially confined cells were then subjected to antifungal treatments (Fig.1b) and the viability of single cells using the viability dye Propidium Iodide (PI) was monitored with time-lapse microscopy (Fig.1c).

The cell's response upon treatment with various concentrations of amphotericin B (AmB), a cell membrane permeabilizing antifungal drug that also induces programmed cell death at certain doses[4], was studied over a period of 6 hours during which viability of the cells was monitored every 15 minutes. We found a dose-dependent increase of the number of PI-positive cells (dead cells) over time. The maximum number of dead cells for each AmB dose was reached after approx. 270 min of incubation (Fig. 2). Treating the cells with higher AmB doses (>50 µM) did not result in 100 % PI-positive staining of cells. Only upon UV treatment, all cells stained PI-positive, indicating complete cell death of the culture. This result indicates that a subpopulation of the yeast culture treated with AmB is PI-negative, indicative for increased AmB tolerance or programmed cell death. Cells dying via programmed cell death or apoptosis are typically characterized by PI-negative staining as the cell membrane is still intact.

In conclusion, **we demonstrate for the first time, an EWOD based DMF device-enabling spatial isolation of single non-adhering yeast cells and their subsequent cytotoxicity read-out upon drug treatment**. Our system allows efficient monitoring of cellular response over a period of time. Research focused on elucidating kinetics of cell responses to drug stimuli and screening for novel antifungals is addressed with this novel digital microfluidic platform.

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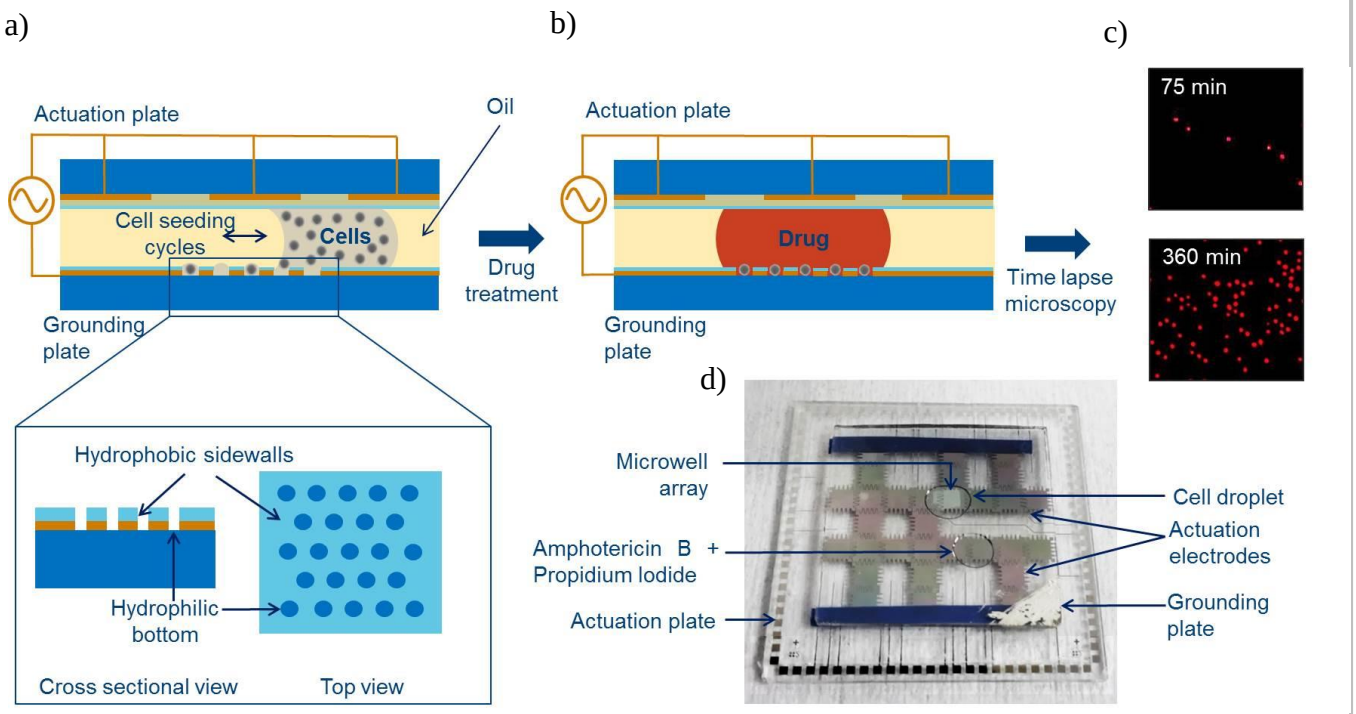

Figure 1: Schematic of the cytotoxicity assay on DMF platform. (a) After assembling the system, the DMF device is flipped upside down and the cell droplet is shuttled back and forth over the microwell array (inset-illustration of fabricated microwells in Teflon® (diameter ~5µm; thickness ~3µm)) in order to trap single cells (b) The cell droplet is removed from the array and the drug droplet containing the viability dye is transported to the trapped cells; (c) a time lapse microscopy is conducted for another 360 minutes to monitor the kinetics of cell viability; (d) Image of the EWOD based DMF two plate assembly.

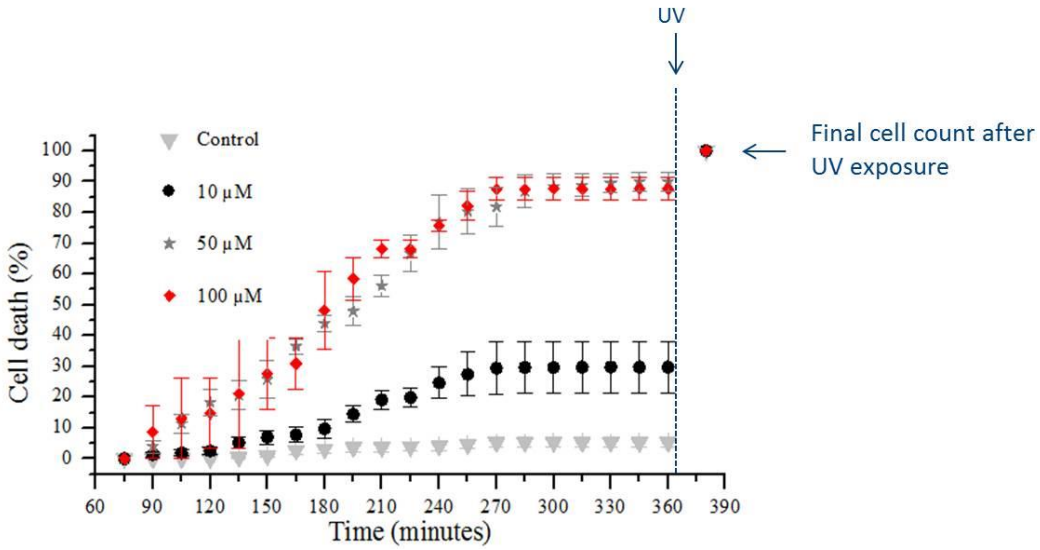

Fig 2: Dose response curve of *S. cerevisiae* cells upon amphotericin B treatments. After 360 minutes, trapped cells were exposed to an intense UV light to kill and count all the trapped cells in fluorescent green excitation wavelength (PI positive staining). Error bars represent a standard error on three replicates.